



**UNIVERSITI PUTRA MALAYSIA**

**LUNG INJURIES INDUCED BY INDEN0(1,2,3-CD)PYRENE IN RATS ON  
GARLIC SUPPLEMENTATION**

**SANAZ MOVASSAGH**

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ON GARLIC SUPPLEMENTATION**

**By**

**SANAZ MOVASSAGH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**April 2007**



*DEDICATED*  
*TO*  
*MY BELOVED PARENTS*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia  
in fulfilment of the requirement for the degree of Doctor of Philosophy

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**Chairman: Associate Professor Noordin Mohamed Mustapha, PhD**

**Faculty : Veterinary Medicine**

The impact of air pollution on health and economic well-being of nation is an important worldwide issue. Indeno[1,2,3-*cd*]pyrene (IP) is a particulate matter amongst environmental pollutants found in the Malaysian haze episodes and was claimed to induce deleterious effects on humans or animals. However, such claims have never been scientifically substantiated. In manifesting the noxious effects of haze and in view of developing strategies and bringing about a remission of such effects in humans, the symptoms of both acute exposure and chronic response to IP were studied on the pulmonary system of rats.

The acute exposure studies were conducted to evaluate the histopathological and ultrastructural changes and detection of apoptotic cells in the lung of rats following treatment with IP with or without garlic supplementation. Furthermore, the immunological responses and elastolytic enzymes activities were also determined. The IP-treated rats received 13 ng (6.5  $\mu$ l) of IP that was instilled intra-tracheally without garlic supplementation. Rats from the garlic (G) group were fed the basal ration

containing garlic incorporated at the rate of 80 mg/kg body-weight/rat/day while those from the G+IP group fed on garlic diet for two weeks before instillation with IP. All animals were sacrificed at 8, 16, 24 and 32 hours (hrs) post-instillation (p.i.) and according to the respective interval design. Histopathological alterations were studied on haematoxylin-eosin (H&E) stained sections and ultrastructural changes revealed by transmission electron microscope (TEM). Apoptosis assessment was made on terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) analysis and caspase 3 colorimetric assay. The broncho-alveolar lavage (BAL) assays, ELISA method and alveolar macrophages (AMØ) activities assay were used to detect the elastolytic enzymes activities and lung defense.

Histopathological, ultrastructural, TUNEL and caspase 3 assays findings showed apoptosis which advances with time in the pneumocytes and bronchial epithelium of all IP-treated rats euthanised at the respective time. In addition, inflammation, necrosis and epithelisation were also observed in the IP group. Likewise, the IP group had the highest elastase-like and neutrophil elastase specific activities but the lowest IgA and IgG levels, AMØ phagocytosis, and intracellular killing activities of AMØ in the BAL. Supplementation with garlic was able to minimize the deleterious effect of IP.

In the chronic response studies, in addition to the other parameters measured in the acute study, an assessment on the level of glutathione S-transferase (GST) was also made. Similarly, there was an additional treatment group that was instilled with IP once at the beginning of the study and fed with garlic during the whole period of experiment (IP+G) to assess the effect of consumption of garlic during the response to IP.

The microscopic and ultrastructure studies revealed evidence of apoptosis, inflammation, necrosis and epithelisation in the lung of rats treated with IP. In addition, rats treated with IP had the lowest levels of IgA, IgG, alveolar macrophages activities and GST specific activity in the lung. Furthermore, there was a dreadful effect on elastolytic enzymes activities present in the IP-treated rats.

In contrast, the IP-treated groups fed with garlic showed significant improvement towards normal histology of the lung or trachea. However, it was found that regularly consumption of garlic during the response to IP have a better result than a prophylactic regime before response.

In conclusion, IP poses as an environmental hazard to the lungs of rats triggering deleterious changes either due to short exposure or long term response and garlic has a great potential in alleviating these adverse effects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KECEDERAAN PARU-PARU TERARUH INDENO(1, 2, 3-CD)PYRENE  
PADA TIKUS YANG MENERIMA TAMBAHAN BAWANG PUTIH**

Oleh

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**April 2007**

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Kesan pencemaran udara pada kesihatan manusia dan status ekonomi negara menjadi salah satu isu penting di seluruh dunia. Indeno[1,2,3-*cd*]pyrene (IP) adalah antara bahan partikel dari pencemar alam sekitar yang ditemui semasa episod jerebu yang melanda Malaysia dan dipercayai boleh mengakibatkan kesan yang merbahaya kepada manusia dan haiwan. Walaubagaimanapun, dakwaan tersebut tidak pernah dibuktikan secara saintifik. Untuk menunjukkan kesan berbahaya jerebu dan untuk menyelidik strategi bagi mengurangkan kesan tersebut pada manusia, simptom-simptom pendedahan akut dan kronik terhadap IP dikaji ke atas sistem pulmonari tikus.

Kajian mengenai pendedahan akut telah dijalankan untuk menilai perubahan histopatologi dan ultrastruktur dan pengenalpastian sel-sel apoptosis pada paru-paru tikus setelah diberi rawatan dengan IP. Selain daripada itu, tindakbalas imunologi dan aktiviti enzim elastolitik telah ditentukan. Tikus dari kumpulan rawatan IP telah diberi 13 ng (6.5  $\mu$ l) IP secara instilasi intra-trakea tanpa suplementasi bawang putih. Tikus dari kumpulan bawang putih (G) diberi makanan komersial dengan penambahan bawang

kadar yang bersamaan dengan pengambilan sebanyak 80 mg/kg berat badan/tikus/sehari manakala tikus dari kumpulan G+IP telah diberi makan diet yang mengandungi bawang putih selama dua minggu sebelum pemberian IP. Kesemua tikus tersebut telah dibunuh pada 8, 16, 24 dan 32 jam (hrs) pasca-pemberian (p.i.) dan mengikut rekabentuk kajian masing-masing. Perubahan secara histopatologikal telah dikaji pada hirisan tisu yang telah diwarnakan dengan haematoxylin-eosin (H&E) manakala perubahan ultrastruktur dikaji dengan menggunakan elektron mikroskop transmisi (TEM). Penilaian apoptosis telah dibuat berdasarkan analisis pelabelan potongan hujung TUNEL dan penilaian caspase 3 kalorimetrik. Penilaian cecair bronko-alveolus (BAL), kaedah ELISA dan aktiviti makrofaj alveolus telah dilakukan untuk mengkaji enzim elastolitik dan pertahanan peparu.

Penemuan histopatologi, ultrastruktur TUNEL dan penilaian caspase 3 menunjukkan tahap apoptosis yang berbeza pada pneumosit and epitelium bronkiol pada semua tikus yang diberi rawatan IP yang dibunuh mengikut jangka masa yang telah ditetapkan pasca pemberian. Di samping itu, inflamasi, nekrosis dan epitelialisasi telah diperhatikan dalam kumpulan IP. Disamping ini, kumpulan IP telah menunjukkan aktiviti spesifik elastase yang tertinggi dan aktiviti spesifik elastase neutrofil tetapi tahap aktiviti yang rendah bagi IgA dan IgG, fagositosis AMØ dan pemusnahan intrasel aktiviti AMØ dalam BAL. Supplementasi dengan bawang putih telah meminimumkan kesan pemusnahan IP.

Dari kajian pendedahan kronik, perubahan pada peparu kumpulan IP selama tiga bulan telah dinilai melalui tahap enzim penanda, glutathion S-transferase (GST). Begitu juga



bagi kumpulan yang diberi IP pada permulaan kajian dan diberi makan bawang putih sepanjang tempoh kajian dijalankan (IP+G) untuk menilai kesan pengambilan bawang putih sepanjang pendedahan terhadap IP.

Dalam kajian mikroskopik dan ultrastruktur menunjukkan bukti apoptosis, inflamasi, nekrosis dan epitelialisasi di dalam paru paru tikus yang dirawat dengan IP. Selain daripada itu, tikus yang diberi IP mempunyai tahap IgA, IgG dan aktiviti makrofaj alveolus serta aktiviti spesifik khas GST dalam paru paru yang paling rendah. Tambahan lagi, terdapat kesan yang dahsyat pada aktiviti enzim elastolisis dalam kumpulan IP.

Sebaliknya, kumpulan yang menerima rawatan IP yang diberi makan bawang putih menunjukkan keadaan yang lebih baik dalam semua perubahan histologi pada paru paru dan trakea. Oleh yang demikian, pengambilan bawang putih yang kerap semasa pendedahan terhadap IP menunjukkan keputusan yang lebih baik berbanding yang menerima pemakanan kurang sebelum pendedahan.

Kesimpulannya, kehadiran IP dalam alam sekitar adalah merbahaya kepada paru-paru tikus yang bertindak sebagai organ target di mana ia mencetuskan perubahan yang merbahaya untuk pendedahan jangka masa pendek mahupun panjang dan didapati bawang putih mempunyai potensi yang amat baik dalam mengurangkan kesan merbahaya tersebut.

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I certify that an Examination Committee has met on 25<sup>th</sup> April 2007 to conduct the final examination of Sanaz Movassagh on her Doctor of Philosophy thesis entitled "Lung Injuries Induced by Indeno(1,2,3-Cd) Pyrene in Rats on Garlic Supplementation" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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**SANAZ MOVASSAGH**

Date: 10 JUNE 2007

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## LIST OF ABBREVIATIONS

Ahr	aryl hydrocarbon receptor
AMØ	alveolar macrophage
ANOVA	analysis of variance
BAL	broncho-alveolar lavage
BALT	bronchial associated lymphoid tissue
BSA	bovine serum albumin
COPD	chronic obstructive pulmonary disease
CRD	completely randomized design
CV	crystal violet
DADS	diallyl disulphide
DAS	diallyl sulphide
DATS	diallyl trisulphide
ELISA	enzyme-linked immunosorbent assay
G	garlic
GSH	glutathione
GST	glutathione S-transferase
H&E	haematoxylin-eosin
hrs	hours
HSD	Tukey's studentized range
IgA	immunoglobulin A
IgG	immunoglobulin G
IP	indeno[1,2,3- <i>cd</i> ]pyrene



OD	optic density
PAHs	polycyclic aromatic hydrocarbons
PBS	phosphate buffered saline
p.i.	post instillation
PM	particulate matter
PMNs	polymorphonuclear leukocytes
pNA	p-nitroaniline
RBC	red blood cell
rER	rough endoplasmic reticulum
rTDT	recombinant terminal deoxynucleotidyl transferase
SAC	S-allyl cysteine
SAMC	S-allyl mercaptocysteine
SAS	statistical analysis system
s.c.	subcutaneous
SE	standard error
sER	smooth endoplasmic reticulum
SLAPN	N-Succinyl-Ala-Ala-Ala-p-nitroanilide
TAMs	tumour associated macrophages
TEM	transmission electron microscopy
TNF $\alpha$	tumor necrosis factor alpha
Tr	Tricaprylin
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling
WR	working reagent